

Comparison of dose responses and resistance ratios in four populations of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), to 20 insecticides

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Abstract

BACKGROUND: Chemical control is a major strategy for suppressing the rice stem borer, *Chilo suppressalis* (Walker). Owing to their high toxicity and increasing resistance development in the target insect, many insecticides will be phased out entirely in 2007 in China. Alternatives with relatively low toxicity are urgently needed to replace traditional chemicals for rice stem borer control. In this study, the authors examined four field populations of *C. suppressalis* for their toxicological responses to more than 20 insecticides, including a few low-toxicity organophosphates and many novel pesticides. Interpopulation resistance levels to 12 conventional insecticides were also compared.

RESULTS: Based on LD₅₀ values, the rice stem borer was most sensitive to avermectins and fipronil (LD₅₀ < 1 ng larva⁻¹). The stem borers exhibited the least sensitivity to endosulfan (LD₅₀ > 100 ng larva⁻¹) and monosultap (LD₅₀ > 1000 ng larva⁻¹). Insect growth regulators and chitin synthase inhibitors showed great efficacy against *C. suppressalis*, especially against populations that had developed resistance to conventional insecticides. Four field populations showed variable tolerance levels to many insecticides. LYG05 was the most susceptible population, only with a low level of resistance to monosultap (RR = 6.6). NC05 and GL05 populations exhibited intermediate tolerance levels with RR values up to 20.4 and 52.8 respectively. RA05 was the most resistant population to many insecticides, with resistance ratios up to 76.2.

CONCLUSION: The results from this study provide valuable information for selection and adoption of new alternative insecticides and for resistance management of the rice stem borer.

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Keywords: *Chilo suppressalis*; resistance; insecticide; alternatives; fipronil; avermectins; IGRs

1 INTRODUCTION

The rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), is one of the economically most important rice insects in China.¹ In the last 10 years, both the pest population density and the intensity of its damage has increased dramatically in China, posing a serious threat to the high and stable yields of the crop.¹ Currently, control of *C. suppressalis* relies mainly on chemical insecticides, especially organophosphates (OPs). Owing to their high toxicity in the environment, some OPs will be banned in 2007 by the Ministry of Agriculture in China. Therefore, a search for effective insecticides with low toxicity to replace highly toxic chemical insecticides was prioritized by the relevant

government authority. Methamidophos, once largely adopted for *C. suppressalis* control, is a listed insecticide for phasing out because of its high toxicity risk. In addition, populations of *C. suppressalis* in many rice regions of China have developed high levels of resistance to monosultap and triazophos, which are two additional conventional insecticides for chemical control of *C. suppressalis*.^{2–16} Resistance to the highly efficacious novel insecticide fipronil has also been observed in some field populations in the last 10 years.^{17–19} Because of these toxicity risks and the increasing development of resistance to conventional insecticides in *C. suppressalis*, it was essential urgently to screen available

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insecticides to find effective alternatives for replacing high-toxicity OPs and other conventional insecticides.

In addition to searching for candidate alternatives for field tests and for selecting additiveness and synergism of insecticide mixtures, this study, in conjunction with the Test and Demonstration Programme for Replacing High-Toxicity Pesticides sponsored by the China Ministry of Agriculture, was carried out to examine tolerance levels to different insecticides in rice stem borers in different rice-growing regions. Field populations were collected in 2005 from four representative regions. Twenty compounds representing seven classes were used to evaluate biological effects on these populations. The resistance ratio (RR) of each field population to individual insecticides was determined by comparing with a laboratory susceptible strain.

2 MATERIAL AND METHODS

2.1 Insects

In 2005, four populations of *C. suppressalis*, LYG05, NC05, GL05 and RA05, were collected from rice paddies in Lianyungang (Jiangsu Province), Nanchang (Jiangxi Province), Guiling District (Guangxi Autonomous Region) and Ruian District (Zhejiang Province) respectively. The locations represented different rice regions in China. All field populations were collected during the egg stage. More than 100 egg masses were collected for each population, and the average size of the egg masses was approximately 100 eggs. Collected insects were reared on rice seedlings by using the protocols of Shang *et al.*²⁰ Fourth-instar larvae were used for bioassays. Rearing conditions were maintained at $28 \pm 1^\circ\text{C}$ and a 16:8 h light:dark photoperiod.

2.2 Insecticides

A total of 20 technical-grade insecticides, listed in Table 1, were used for bioassays on *C. suppressalis*. These insecticides represented seven classes including macrocyclic lactone antibiotic insecticides (avermectins: abamectin and emamectin benzoate), a phenylpyrazole insecticide (fipronil), 11 organophosphates (OPs), insect growth regulators (IGRs functioning as ecdysteroid agonists: tebufenozide and JS118), chitin synthase inhibitors (benzoylphenyl ureas: hexaflumuron and chlorfluazuron), an organochlorine (endosulfan) and a nereis-toxin analogue (monosultap).

2.3 Bioassays

A topical application method²¹ was used to conduct bioassays on each population of *C. suppressalis*. Larvae at the middle of the fourth instar with a body weight ranging from 6 to 9 mg larva⁻¹ were used as standard test larvae, as recommended by Cao *et al.*⁷

Larvae were placed into petri dishes containing a piece of artificial diet (1 × 1 × 0.3 cm) based on the components uses by Tan,²² which were revised

from the recipe of the FAO.²¹ Insecticides were dissolved in acetone at a series of concentrations (5–7 concentrations plus a control), except monosultap for which a mixture of acetone and water at a ratio of 1 + 1 (by volume) was used because of its low solubility in acetone. A droplet (0.04 µL) of insecticide solution was applied topically to the dorsal part of the larval middle abdomen using a capillary microapplicator. Three replicates were used, and a total of 30 larvae were treated for each insecticide concentration. Control insects were treated with acetone only, or with a mixture of acetone and water as control for monosultap treatments. After treatment, the larvae were maintained at $28 \pm 1^\circ\text{C}$ and 16:8 h light:dark. Mortality was recorded 48 h after treatment for OPs and organochlorine, 72 h for fipronil, 96 h for avermectins and monosultap and 144 h for insect growth regulators (IGRs) and benzoylphenyl ureas. The time length for post-treatment mortality counting was based on the effectiveness of each insecticide to allow a good range of mortality rates for the LD₅₀ calculation. Larvae were counted as dead if no response was obtained after being probed with a pin.

2.4 Statistical analysis

PoloPlus software²³ was used for probit analysis of dose–response data and calculations of LD₅₀ values. The resistance ratio (RR) for the four field populations was calculated by dividing the LD₅₀ of the field populations by the corresponding LD₅₀ of a susceptible strain Hwc-S. The susceptible strain Hwc-S was originally collected in 2002 from Wuchang city, Heilongjiang Province, and cultured successively in the lab without any pesticide treatments. Susceptibility or tolerance levels were classified on the basis of the standard of Shen and Wu²⁴ as: susceptible – RR < threefold; minor resistance – RR = 3–5-fold; low resistance level – RR = 5–10-fold; medium resistance level – RR = 10–40-fold; high resistance level – RR = 40–160-fold; extremely high resistance level – RR > 160-fold. Data were statistically analysed with the SAS program,²⁵ and Proc Mixed and Proc GLM procedures were used for variance analyses. Mean separation was conducted using SAS Proc Means/LSD and LSMeans procedures at $P < 0.05$.

3 RESULTS

3.1 Variation of dose responses among four populations

Two field populations, LYG05 and RA05, were used to test dose responses to 20 insecticides. Two other populations, NC05 and GL05, were used for testing 17 insecticides (chlorfluazuron, hexaflumuron and endosulfan were excluded owing to insufficient numbers being collected). Statistics analysis using the Proc mixed model indicated that the interaction between population and insecticide was significant ($F_{51,148} = 27.23$, $P < 0.0001$). The dose responses

Table 1. Details of 20 insecticides used for assaying dose responses and resistance ratios in four field populations of *Chilo suppressalis*

Insecticide	Class	Technical grade (a.i.)	Company
Abamectin	Antibiotic	93% B1a	Hebei Veyong Bio-chemical Co., Ltd
Emamectin benzoate	Antibiotic	89% B1a	Hebei Veyong Bio-chemical Co., Ltd
Fipronil	Phenylpyrazole	87%	Bayer CropScience Hangzhou Co., Ltd
JS118	Ecdysteroid agonist	97%	Nanjing Suyan Kechuang Agrochemical Co., Ltd
Tebufenozide	Ecdysteroid agonist	95%	Jiangsu Baoling Chemical Co., Ltd
Chlorfluazuron	Benzoylphenyl urea	98.1%	Jiangsu Yangnong Chemical Co., Ltd
Hexaflumuron	Benzoylphenyl urea	97.1%	Jiangsu Yangnong Chemical Co., Ltd
Acephate	Low-toxicity organophosphate	98.3%	Jiangsu Suhua Group Co., Ltd
Chlorpyrifos	Low-toxicity organophosphate	95%	Nantong Jiangshan Agrochemical & Chemicals Co., Ltd
Diazinon	Medium-toxicity organophosphate	95%	Nantong Jiangshan Agrochemical & Chemicals Co., Ltd
Fenitrothion	Low-toxicity organophosphate	95.0%	Zhejiang Yongning Chem. Ind. Co., Ltd
Malathion	Low-toxicity organophosphate	94.5%	Henshui-NPC Co., Ltd
Methamidophos	High-toxicity organophosphate	73%	Shandong Huayang Technology Co., Ltd
Phoxim	Low-toxicity organophosphate	89%	Jiangsu Baoling Chemical Co., Ltd
Pyridaphenthion	Low-toxicity organophosphate	88.5%	Anhui Chizhou Sincerity Chemicals Co., Ltd
Quinalphos	Medium-toxicity organophosphate	70%	Zhejiang Jiaxing Jiahua Group Co., Ltd
Triazophos	Medium-toxicity organophosphate	88%	Jiangsu Baoling Chemical Co., Ltd
Trichlorphon	Low-toxicity organophosphate	90%	Institute for the Control of Agrochemicals, Ministry of Agriculture
Monosultap	Nereistoxin analogue	90%	Jiangsu Liyang Chemical Factory
Endosulfan	Organochlorine	93%	Danyang Agrochemicals Co., Ltd

of each population to selected insecticides are summarized in Table 2.

LD₅₀ values of the LYG05 population to the 20 insecticides ranged from 0.16 to 1866 ng larva⁻¹ (Table 2). Results indicated that the LYG05 population showed the most susceptibility to three insecticides, emamectin benzoate, abamectin and fipronil, and the LD₅₀ values were all below 1 ng larva⁻¹. The population was very sensitive to phoxim, quinaphos, chlorpyrifos, triazophos, JS118, pyridaphenthion and tebufenozide, with LD₅₀ values lower than 10 ng larva⁻¹. Four insecticides, trichlorphon, endosulfan, acephate and monosultap, were less efficacious against the stem borer, especially monosultap which had the highest LD₅₀ value of 1866 ng larva⁻¹.

LD₅₀ values to the 17 selected insecticides in the NC05 population ranged from 0.04 to 3030 ng larva⁻¹ (Table 2). Similarly, emamectin benzoate, abamectin and fipronil were the most effective insecticides against *C. suppressalis*, with LD₅₀ values all below 1 ng larva⁻¹. Monosultap and methamidophos were the least effective pesticides against the insect, with LD₅₀ values of 3030 and 562 ng larva⁻¹ respectively.

For the GL05 population, 17 insecticides showed a toxicological effect (LD₅₀): emamectin benzoate > abamectin > fipronil > quinaphos > JS118 > phoxim > tebufenozide > chlorpyrifos > pyridaphenthion > malathion > fenitrothion > diazinon > acephate > triazophos > methamidophos > trichlorphon > monosultap. The LD₅₀ values of these 17 selected insecticides ranged from 0.1 to 3308 ng larva⁻¹ (Table 2).

For the RA05 population, 20 insecticides showed a toxicological effect (LD₅₀): emamectin benzoate > abamectin > fipronil > tebufenozide > chlorfluazuron > JS118 > phoxim > hexaflumuron > quinaphos > pyridaphenthion > diazinon > chlorpyrifos > malathion > fenitrothion > triazophos > methamidophos > acephate > trichlorphon > endosulfan > monosultap. LD₅₀ values to these 20 insecticides ranged from 0.14 to 15 661 ng larva⁻¹ (Table 2).

Pooled data from all 20 insecticides (Fig. 1) showed that four field populations had significantly different mean dose responses to the insecticides tested ($F_{3,218} = 5.45$, $P < 0.01$). The LYG05 population had the lowest mean LD₅₀ (131 ng larva⁻¹). The

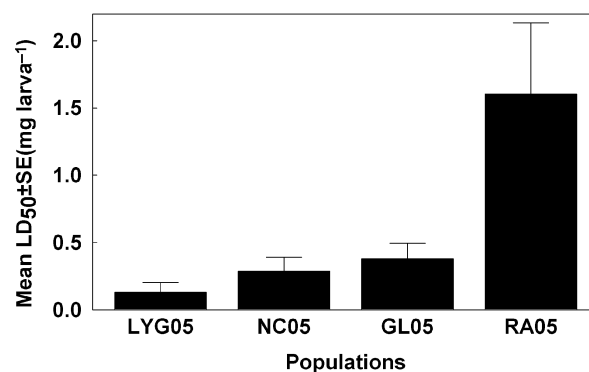


Figure 1. Dose responses (pooled LD₅₀s from 20 insecticides) among four field populations of *C. suppressalis* collected in 2005 from four provinces (LYG05: Lianyungang, Jiangsu; NC05: Nanchang, Jiangxi; GL05: Guilin, Guangxi; RA05: Ruian, Zhejiang).

Table 2. Dose responses ($LD_{50} \pm SE$)^a to 20 insecticides in four field populations of *Chilo suppressalis* (LYG05: Lianyungang, Jiangsu; NC05: Nanchang, Jiangxi; GL05: Guiling, Guangxi; RA05: Ruian, Zhejiang)

Insecticides	LYG05	NC05	GL05	RA05
Abamectin	0.24 (± 0.04) b	0.04 (± 0.01) c	0.47 (± 0.02) f	0.33 (± 0.02) c
Emamectin benzoate	0.16 (± 0.01) b	0.07 (± 0.01) c	0.10 (± 0.01) f	0.14 (± 0.02) c
Fipronil	0.52 (± 0.13) b	0.63 (± 0.08) c	0.83 (± 0.05) f	9.93 (± 0.41) c
JS118	7.27 (± 0.19) b	11.40 (± 1.00) c	26.8 (± 2.00) ef	16.3 (± 2.49) c
Tebufenozide	7.53 (± 3.16) b	12.70 (± 1.65) c	40.5 (± 16.5) def	12.3 (± 1.32) c
Chlorfluazuron	15.3 (± 2.70) b	– ^b	– ^b	12.7 (± 3.59) c
Hexaflumuron	27.7 (± 5.85) b	– ^b	– ^b	41.1 (± 3.32) c
Acephate	201 (± 23.9) b	336 (± 32.8) bc	310 (± 18.7) cde	913 (± 96.8) c
Chlorpyrifos	5.07 (± 0.43) b	15.3 (± 0.98) c	89.7 (± 9.23) def	240 (± 34.2) c
Diazinon	14.7 (± 2.12) b	51.3 (± 6.85) c	167 (± 48.3) cdef	170 (± 27.4) c
Fenitrothion	14.3 (± 2.40) b	43.8 (± 5.83) c	118 (± 15.2) cdef	418 (± 38.5) c
Malathion	16.9 (± 0.81) b	179 (± 27.1) bc	108 (± 15.8) cdef	345 (± 28.6) c
Methamidophos	95.8 (± 7.43) b	562 (± 71.1) b	391 (± 58.7) c	793 (± 96.3) c
Phoxim	2.67 (± 0.82) b	8.90 (± 0.87) c	33.0 (± 3.81) def	26.4 (± 2.22) c
Pyridaphenthion	7.50 (± 0.20) b	42.2 (± 3.13) c	105 (± 13.8) cdef	157 (± 16.9) c
Quinalphos	4.07 (± 0.67) b	11.2 (± 1.98) c	23.4 (± 2.67) ef	54.0 (± 4.85) c
Triazophos	6.83 (± 1.42) b	127 (± 11.9) bc	328 (± 66.4) cd	473 (± 86.2) c
Trichlorphon	123 (± 10.8) b	446 (± 41.2) bc	1382 (± 129) b	1730 (± 51.02) c
Monosulap	1866 (± 804) a	3030 (± 648) a	3308 (± 393) a	15 661 (± 2544) a
Endosulfan	196 (± 4.93) b	– ^b	– ^b	10 994 (± 974) b

^a Means followed by the same letters are not significantly different at $P = 0.05$ within a column.

^b Experiments were not conducted owing to limited insect collections.

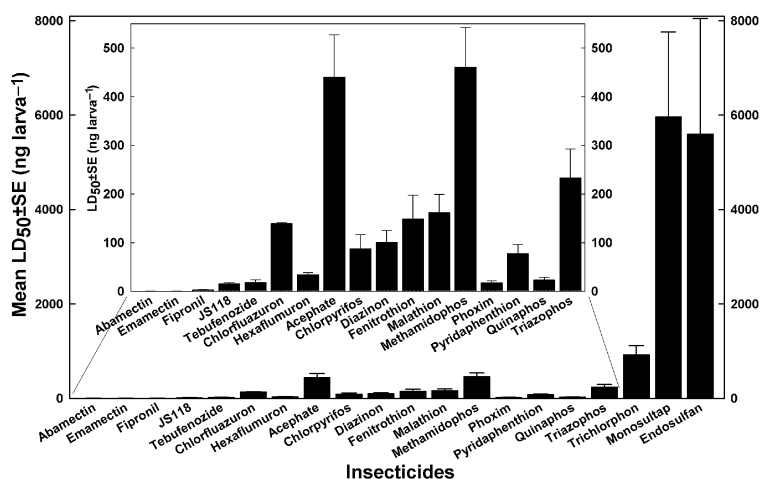
NC05 and GL05 populations had mean LD_{50} values of 287 and 378 ng larva⁻¹ respectively. The RA05 population had the highest mean LD_{50} (1603 ng larva⁻¹), which was significantly different from those of the other three populations ($P < 0.0001$).

3.2 Comparison of toxicological effects among 20 insecticides

Pooled LD_{50} data from four field populations indicated significantly different responses of *C. suppressalis* to 20 insecticides ($F_{19,202} = 9.44$, $P < 0.0001$) (Fig. 2) of seven insecticide classes ($F_{6,215} = 30.87$, $P < 0.0001$). The insects were most sensitive to emamectin benzoate ($LD_{50} = 0.12$ ng larva⁻¹) and abamectin ($LD_{50} = 0.27$ ng larva⁻¹) and least sensitive

to endosulfan ($LD_{50} = 5595$ ng larva⁻¹) and monosulap ($LD_{50} = 5966$ ng larva⁻¹).

The insects were also very sensitive to fipronil ($LD_{50} = 2.98$ ng larva⁻¹), chlorfluazuron ($LD_{50} = 14.0$ ng larva⁻¹), JS118 ($LD_{50} = 15.4$ ng larva⁻¹), tebufenozide ($LD_{50} = 18.3$ ng larva⁻¹) and hexaflumuron ($LD_{50} = 34.4$ ng larva⁻¹). The insects showed significantly different responses to 11 organophosphates ($F_{10,120} = 12.86$, $P < 0.0001$). Pooled LD_{50} values ranged from 18 ng to 920 ng per insect. Among 11 organophosphates, phoxim ($LD_{50} = 17.7$ ng larva⁻¹) and quinalphos ($LD_{50} = 23.2$ ng larva⁻¹) were the most effective insecticides, while trichlorphon ($LD_{50} = 920$ ng larva⁻¹), methamidophos ($LD_{50} = 460$ ng larva⁻¹) and acephate ($LD_{50} =$

**Figure 2.** Differential dose response (pooled LD_{50} s from four populations, LYG05, NC05, GL05 and RA05) of *C. suppressalis* to 20 insecticides (inset shows large scale of LD_{50} for 17 insecticides).

440 ng larva⁻¹) were the least effective. The other six organophosphates had intermediate effectiveness (LD₅₀ values from 78 to 233 ng larva⁻¹) against *C. suppressalis*.

3.3 Analysis of resistance ratios among four populations

The baselines of *C. suppressalis* to 12 conventional insecticides of six insecticide classes were developed by using a laboratory susceptible strain (Hwc-S) in 2002. Based on the susceptible baselines, the resistance ratios of the four field populations to these 12 conventional insecticides were determined. Results showed that NC05 ($F_{10,22} = 43.89$, $P < 0.0001$), GL05 ($F_{10,22} = 19.21$, $P < 0.0001$) and RA05 ($F_{11,24} = 24.74$, $P < 0.0001$) populations had developed significant resistance to many insecticides, while LYG05 ($F_{11,24} = 4.18$, $P < 0.005$) was susceptible to most of these tested insecticides but had developed a low level of resistance to monosultap (RR = 6.55).

Based on Shen and Wu's standard,²⁴ the NC05 population maintained susceptibility to the seven tested insecticides with RR values below 3 (Table 3). This population developed a minor level of resistance (RR = 3–5) to fenitrothion, a low level of resistance (RR = 5–10) to trichlorphon and a medium level of resistance (RR = 10–40) to monosultap (RR = 10.6) and triazophos (RR = 20.4).

The GL05 population was susceptible to acephate, fipronil, tebufenozide and abamectin (Table 3). It had a minor level of resistance to diazinon and a low level of resistance to phoxim. A medium level of resistance was observed to chlorpyrifos, monosultap, fenitrothion and trichlorphon. A high level of resistance was observed to triazophos (RR = 52.8) in the GL05 population.

Although the variance analysis indicated a high tolerance level in the RA05 population, the population still maintained susceptibility to abamectin, tebufenozide and acephate. It had a minor level of resistance to diazinon and a low level of resistance

to phoxim and fipronil. A medium level of resistance to trichlorphon, chlorpyrifos and fenitrothion was detected in the population. A high level of resistance was seen not only to triazophos (RR = 76.2), as observed in the GL05 population, but also to monosultap (RR = 55.0) and endosulfan (RR = 55.3) (Table 3).

Pooled data from all 12 insecticides (Fig. 3) showed that four field populations had significantly different resistance ratios to the insecticides tested ($F_{3,134} = 16.83$, $P < 0.0001$). Compared with the susceptible strain (Hwc-S), the LYG05 population was very susceptible, with a mean RR of 1.36 ± 0.35 , and the NC05 population had the second lowest mean RR (4.49 ± 1.02). The GL05 population had tenfold (11.4 ± 2.65) tolerance, while the RA05 population had the highest RR (25.8 ± 4.42), which was significantly different from those of the other three populations ($P < 0.05$).

3.4 Analysis of resistance ratio variations among 12 insecticides

Pooled RRs from the four field populations showed that *C. suppressalis* developed significantly different

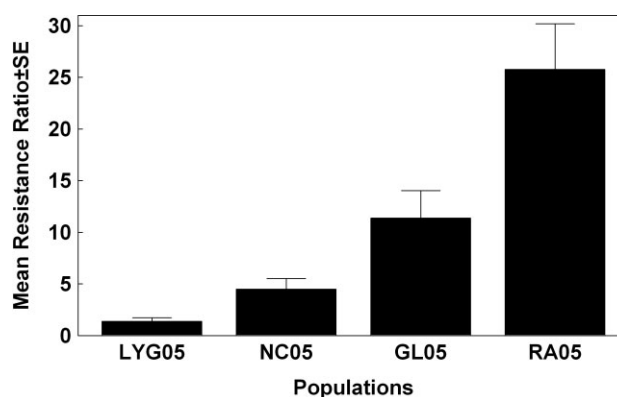


Figure 3. Resistance ratios (pooled RRs from 12 insecticides) among four field populations of *C. suppressalis* collected in 2005 from four provinces (LYG05: Lianyungang, Jiangsu; NC05: Nanchang, Jiangxi; GL05: Guiling, Guangxi; RA05: Ruian, Zhejiang).

Table 3. Differential resistance ratios [RR (± SE)]^a to 12 insecticides among four field populations of *Chilo suppressalis* (LYG05: Lianyungang, Jiangsu; NC05: Nanchang, Jiangxi; GL05: Guiling, Guangxi; RA05: Ruian, Zhejiang)

Insecticides	LYG05	NC05	GL05	RA05
Abamectin	1.43 (±0.24) b	0.21 (±0.03) d	2.76 (±0.09) de	1.94 (±0.12) e
Fipronil	0.52 (±0.13) b	0.63 (±0.08) d	0.83 (±0.05) e	9.93 (±0.40) e
Tebufenozide	0.49 (±0.21) b	0.82 (±0.11) d	2.63 (±1.07) de	0.80 (±0.09) e
Acephate	0.48 (±0.06) b	0.80 (±0.08) d	0.74 (±0.44) e	2.18 (±0.23) e
Chlorpyrifos	0.60 (±0.05) b	1.82 (±0.12) d	10.7 (±1.10) bcde	28.6 (±4.07) c
Diazinon	0.36 (±0.05) b	1.27 (±0.17) d	4.12 (±1.19) cde	4.20 (±0.68) e
Fenitrothion	1.55 (±0.26) b	4.76 (±0.64) c	12.8 (±1.65) bc	45.5 (±4.19) b
Phoxim	0.58 (±0.18) b	1.93 (±0.19) d	7.17 (±0.83) cde	5.75 (±0.48) e
Triazophos	1.10 (±0.23) b	20.4 (±1.93) a	52.8 (±10.71) a	76.2 (±13.9) a
Trichlorphon	1.68 (±0.14) b	6.08 (±0.32) c	18.8 (±1.76) b	23.6 (±0.69) cd
Monosultap	6.55 (±2.82) a	10.6 (±2.27) b	11.6 (±1.38) bcd	55.0 (±8.93) b
Endosulfan	0.99 (±0.02) b	– ^b	– ^b	55.4 (±4.91) b

^a Means followed by the same letters are not significantly different at $P = 0.05$ within a column.

^b Experiments were not conducted owing to limited insect collections.

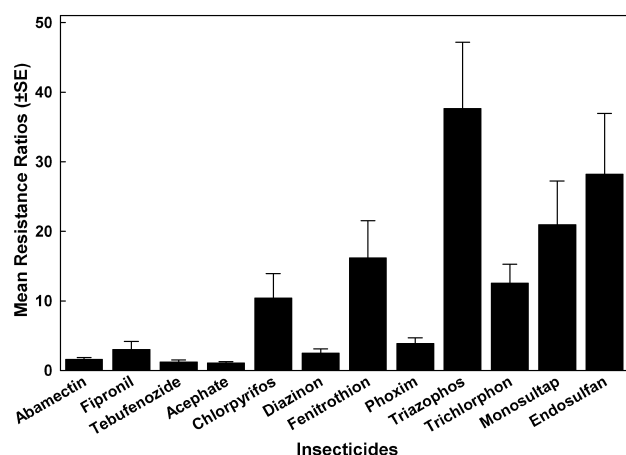


Figure 4. Variable resistance ratios of *C. suppressalis* to 12 insecticides (pooled RRs from four populations, LYG05, NC05, GL05 and RA05).

tolerance levels (RRs) to 12 individual insecticides ($F_{11,126} = 7.04$, $P < 0.0001$) of six insecticide classes ($F_{5,132} = 3.99$, $P < 0.005$). The insect maintained susceptibility and had a minor level of resistance to tebufenozide (RR = 1.19 ± 0.35), abamectin (RR = 1.59 ± 0.29), fipronil (RR = 2.98 ± 1.21) and a few OP insecticides such as acephate (1.05 ± 0.21), diazinon (2.49 ± 0.59) and phoxim (3.86 ± 0.84). Low levels of resistance were detected for chlorpyrifos (RR = 10.4 ± 3.49), trichlorphon (12.5 ± 2.73) and fenitrothion (RR = 16.2 ± 5.34). Medium levels of resistance were observed in the insect for monosultap (RR = 20.9 ± 6.30), endosulfan (RR = 28.2 ± 8.74) and triazophos (RR = 37.7 ± 9.50) (Fig. 4).

4 DISCUSSION

In this study, twenty insecticides were examined for their activity against four field populations of *C. suppressalis*. These insecticides represented seven different insecticide classes. Based on the LD₅₀ and resistance ratio data, the authors suggest that many of the tested insecticides are potential candidates for replacing high-toxicity pesticides. These potential alternatives include some novel insecticides (avermectins and fipronil), insect growth regulators and a few organophosphates with high efficacy against the insect and low toxicity to the environment.

Fipronil, a novel phenylpyrazole insecticide, has been applied extensively in rice fields for the control of major rice pest insects, including *C. suppressalis*, since 1997.²⁶ Cao²⁷ found no cross-resistance between fipronil and conventional insecticides in *C. suppressalis*. Hence, fipronil is considered a leading alternative for replacing highly toxic pesticides such as triazophos, to which *C. suppressalis* has developed high-level resistance.^{3–5,13,14} However, some field populations of *C. suppressalis* have developed medium levels of resistance to fipronil.^{17–19} The present data indicated that only the RA05 population had developed approximately 9.9-fold tolerance, while the

three other populations, LYG05, NC05 and GL05, still maintained susceptibility to fipronil. Further investigations are needed to verify and to monitor the tolerance development in different regions. Resistance management strategies need to be developed to maintain susceptibility in the target insect.

Avermectins are a group of antibiotic (macrocyclic lactone) pesticides with broad-spectrum and high efficiency.²⁸ Many mixtures of abamectin have been registered for controlling rice borers and have been applied for the control of *C. suppressalis* since 1998 in China.^{8,29} Emamectin benzoate (Proclaim) is a novel member of the avermectin family and was obtained from abamectin via a five-step synthesis.²⁹ Results showed that avermectins were the most effective chemical group against *C. suppressalis* among the 20 compounds tested, and emamectin benzoate had a slightly higher efficacy than abamectin. The present data were similar to the toxicity results reported by Ni *et al.*³⁰ Therefore, avermectins are also considered ideal alternatives for the control of *C. suppressalis*, including the RA05 population which has medium to high tolerance to many insecticides.

The present data indicated that IGRs are relatively effective against *C. suppressalis* when compared with other insecticide classes. They are potential candidates for managing populations, especially those with high tolerance levels to OPs and other insecticides. Insect growth regulators have often been called third-generation insecticides³¹ owing to their different mode of action against insect pests. Tebufenozide (RH5992) is a bisacylhydrazine ecdysteroid agonist that is effective against lepidopteran pests.³² This compound was recommended by Rohm-Haas Company (Pennsylvania, USA) to control many lepidopteran insects in vegetables, rice, fruit, etc., and is used to control rice stem borer in Spain, where its efficacy was similar to that of the conventional insecticides for the control of stem borers.³³ In China, it is only used to control vegetable pests and has not been applied in rice fields.²⁶ JS118 is a novel bisacylhydrazine ecdysteroid agonist discovered by the Branch of National Pesticide Research & Development South Center in China.⁸ The present data indicate that the efficacy of JS118 is similar to that of tebufenozide, in agreement with the results of Cao *et al.*⁸ For the relatively susceptible population LYG05, tebufenozide and JS118 were less effective than phoxim and quinaphos, and, for the NC05 population, tebufenozide and JS118 had similar efficacies to phoxim and quinaphos. For the RA05 population with high levels of resistance to triazophos, chlorpyrifos and endosulfan, the two ecdysteroid agonists had higher efficacies than phoxim and quinaphos. Data indicated that all four field populations, LYG05, NC05, GL05 and RA05, were susceptible to tebufenozide. In spite of their slow action against insects, their unique mode of action makes IGRs a potential alternative to be rotated or mixed with other fast-acting insecticides.

Hexaflumuron and chlorfluazuron, the benzoylphenyl ureas (BPUs), act on insects by inhibiting chitin formation.³⁴ BPUs generally affect the larval stages of insects when chitin is synthesized during moulting. Therefore, the adults of beneficial species, such as predators and parasitoids, are seldom affected.³⁵ Hexaflumuron and chlorfluazuron were less effective than diazinon against the LYG05 population but exhibited higher efficacy than diazinon against the RA05 population.

Ten low- or medium-toxicity OP insecticides were selected to examine their efficacies against *C. suppressalis*. Based on LD₅₀ data, phoxim, chlorpyrifos, quinaphos, pyridaphenthion and diazinon are potential alternatives for many regions because they have a relatively high efficacy against *C. suppressalis*. However, the RA05 population showed a medium level of resistance to chlorpyrifos owing to its extensive use in rice fields. Therefore, application of chlorpyrifos should be limited in some rice areas.

Variable tolerance levels were detected in four selected populations of *C. suppressalis*. The populations represented different rice-growing regions, including the eastern region for the LYG05, the south-eastern region for the NC05 (Jianxi Province) and RA05 (Zhejiang Province) and the southern region of China for the GL05 population. Variable insecticide application levels may have contributed to the tolerance development in different regions. In the region (Lianyungang, Jiangsu Province) where the LYG05 population was collected, the application level of insecticides is relatively low, and the history of insecticide usage in rice fields is very short. Although the density of this population has been increasing in recent years, only monosultap was used during the past 5 years in rice fields in this area.¹⁷ Therefore, the LYG population was very susceptible to almost all the tested insecticides except for a low level of resistance to monosultap. In the south-eastern region, especially where the RA population was collected for this study, the rice stem borer produces four generations a year. Pesticides are applied more than 5 times a year, which is the highest level of pesticide application in China for the control of this pest. The resistance level was the highest among the four populations investigated in this study. The results were consistent with observations from other resistance monitoring programmes conducted in the past 5 years with many field populations (He *et al.*, unpublished data), indicating that the tolerance level to almost all of the conventional insecticides in the field populations from Wenzhou district, including the RA region, was the highest level among all the tested field populations in China. The NC and GL populations were collected from two major rice production regions, representing the middle and lower stream areas of the Yangzi River and the southern rice production regions respectively. In these regions, the application levels of insecticides are relatively high, although not as high as the levels in the RA region. The authors' monitoring programme

indicated that the two populations have also developed moderate resistance to triazophos.

It is likely that the intensity of insecticide application correlated with the resistance development in *C. suppressalis* that was found in this study. The lowest resistance ratios were detected from the region (LYG) with the lowest level of insecticide applications, while the highest resistance ratios were associated with the highest level of insecticide applications in the RA population. Limited comparisons of LD₅₀ values of triazophos between 2004 and 2005 (manuscript in submission) indicated that the susceptibility of the LYG population was relatively stable. The LYG population also maintained susceptibility levels to most of the conventional insecticides. However, the RA population consistently showed higher levels of resistance ratios to triazophos in both 2004 and 2005. Limited data (He *et al.*, unpublished data) also indicated a rapid resistance increase in the RA population over the years 2004 and 2005. This phenomenon necessitates studies to investigate the nature of resistance, resistance gene frequency and distribution in the fields, and how to prevent or slow down resistance development.

In summary, dose responses of multiple populations to 20 insecticides were examined in this study. The results provided not only toxicological information of individual insecticides against an economically important insect but also background information on resistance development in four different regions. Analysis of LD₅₀ values revealed different efficacy levels for all 20 insecticides, which can be used to accelerate screening and selection of potential alternatives for replacing highly toxic organophosphate insecticides that are currently used for rice stem borer control but are to be phased out soon. Comparison of tolerance levels in four populations revealed a potential risk of resistance development to certain insecticides, and provided the information for strategic development to achieve effective chemical control of *C. suppressalis* with minimal risk of toxicity to the environment and minimal risk of resistance development in the target insect.

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